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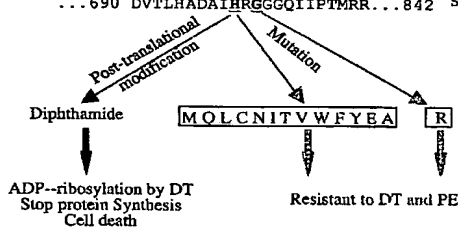
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- (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by the SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH [US/US]; Office of Technology Transfer 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).
- (72) Inventors; and
(75) Inventors/Applicants (for US only): NEVILLE, David, M. [US/US]; 9624 Parkwood Drive, Bethesda, MD 20814 (US). WOO, Jung-Hee [KR/US]; 259 Congressional Lane, Apt. 214, Rockville, MD 20852 (US). LIU, Yuan-Yi [CN/US]; 8415 Buckhannon Drive, Potomac, MD 20854 (US).
- (74) Agents: SPRATT, Gwendolyn, D. et al.; Needle & Rosenberg, P.C., Suite 1000, 999 Peachtree Street, Atlanta, GA 30309-3915 (US).
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(54) Title: METHODS FOR EXPRESSION AND PURIFICATION OF IMMUNOTOXINS

<i>H. sapiens</i> (66%)	...706	DVTLHADAIHRGGGQIIPTARR...	858	SEQ. ID NO:1
<i>M. musculus</i> (3'mRNA)	DVTLHADAIHRGGGQIIPTARR...		SEQ ID NO:2
<i>R. norvegicus</i> (66%)	...706	DVTLHADAIHRGGGQIIPTARR...	858	SEQ ID NO:3
<i>C. griseus</i> (CHO, 67%)	...706	DVTLHADAIHRGGGQIIPTARR...	858	SEQ ID NO:4
<i>D. melanogaster</i> (67%)	...692	DVTLHADAIHRGGGQIIPTARR...	848	SEQ ID NO:5
<i>C. elegans</i> (67%)	...700	DVTLHADAIHRGGGQIIPTARR...	852	SEQ ID NO:6
<i>S. pombe</i> (79%)	...690	DVTLHADAIHRGGGQIIPTARR...	842	SEQ ID NO:7
<i>P. pastoris</i> (88%)	...690	DVTLHADAIHRGGGQIIPTARR...	842	SEQ ID NO:8
<i>S. cerevisiae</i>	...690	DVTLHADAIHRGGGQIIPTARR...	842	SEQ ID NO:9



(57) Abstract: In one aspect the present invention relates to a method of expressing an immunotoxin in *Pichia pastoris* strain mutated to toxin resistance comprising a) growing the *Pichia pastoris* in a growth medium comprising an enzymatic digest of protein and yeast extract and maintaining a dissolved oxygen concentration at 40% and above; and b) performing methanol induction with a limited methanol feed of 0.5-0.75 ml/min/ 10L of initial volume during induction along with a continuous infusion of yeast extract at a temperature below 17.5 °C., antifoaming agent supplied up to 0.07%, agitation reduced to 400 RPM, and the induction phase extended out to 163 h. In another aspect, the present invention relates to a method of purifying a nonglycosylated immunotoxin comprising a) loading a solution containing the nonglycosylated immunotoxin onto a hydrophobic interaction column; b) obtaining a first non-glycosylated immunotoxin containing eluant from the hydrophobic interaction column; c) loading the non-glycosylated immunotoxin containing eluant from step (b) onto an anion exchange column; d) obtaining a second

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GAT GTT ACC CTG CAC GCC GAT GCT ATC CAC AGA GGT GGA GGA CAA GTC ATT CCA ACC ATG AAG AGA

D V T L H A D A I H R G G G Q I I P T A R R ... 700 ... 710

SEQ ID NO:10

non-glycosylated immunotoxin containing eluant from the anion exchange column by eluting the non-glycosylated immunotoxin with a sodium borate solution; e) diluting the concentration of sodium borate in the second non-glycosylated immunotoxin containing eluant from step (d) to about 50 mM or less; f) concentrating the diluted non-glycosylated immunotoxin containing eluant from step (e) over an anion exchange column; and g) obtaining a purified non-glycosylated immunotoxin from the anion exchange column.



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